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13. ABSTRACT (Maximum 200 words) The primary objectives of this research were to determine the capabilities of ESI-IMS for monitoring of aqueous samples containing chemical warfare agents or their subsequent degradation products. Rapid analysis times, excellent sensitivity, and potential portability make IMS an excellent tool for field applications. The ion chemistry for several chemical warfare degradation products were studied both with a prototype instrument and a high-resolution ESI-IMS instrument coupled with a quadrupole mass spectrometer (MS), showing excellent sensitivities for these compounds. Also, the ability to directly monitor water samples without prior sample preparation was shown. A portable ESI-IMS instrument has been designed and constructed. A high resolution ESI-IMS instrument coupled with a time of flight MS was assembled and showed excellent sensitivities for several chemical warfare degradation compounds. Due to the time compatibility of IMS and TOFMS, nine compounds were all resolved in two dimensions (mobility and m/z) within a minute.				
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Enclosure 1

QUANTITATIVE AND QUALITATIVE DETERMINATION OF ORGANICS IN WATER USING ELECTROSPRAY IONIZATION COUPLED WITH ION MOBILITY SPECTROMETRY

Final Progress Report

15 Mar 98 – 14 Sep 02

Agreement No. DAAG55-98-1-0107

Submitted to:

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STATEMENT OF THE PROBLEM STUDIED

The number of toxic or infectious agents that can be used in a military or civilian environment is quite large. These materials range from simple organic chemicals to complex bioengineered microorganisms. At one end of the spectrum are vapor phase nerve agents that will kill nearly instantly. In the middle are biochemicals such as neuroactive peptides that have low vapor pressure but have extremely disruptive effects on human life. At the other end are infectious bacterial agents that will kill in a dose of only one organism but with a longer onset time. Unfortunate examples of agent use and seizures include:

The recent incidence of what is thought to be terrorist outbreaks of anthrax distribution through the United Postal Service,

138 people dead from anthrax in Sverdlovsk (now Ekaterinburg), Russia,

12 people dead from sarin in Tokyo, Japan,

The intentional Salmonella poisoning of 751 people in Oregon, and

Seizures of anthrax vaccine, bubonic plague cultures, and ricin from militia organizations within the United States.

These incidents indicate that a growing threat of attack on both military and civilian populations is imminent. However, even the most highly prepared combat force will not be able to positively detect an attack of either biological or chemical warfare agents until roughly about 25 to 40 minutes after it has been initiated. This critical window of vulnerability needs to be closed by the development of a rapid field-portable detection method to include detection of the wide range of plausible agents.

The goal of this project was to evaluate a novel chemical analysis method called electrospray ionization (ESI) with ion mobility spectrometry (IMS) for agent detection in aqueous samples.

Commercial IMS instruments are currently utilized for several applications such as explosives, drugs and chemical warfare agents. Their widespread popularity has been due to their portability, rapid response, and excellent sensitivity. However, current commercial IMS instruments are limited to analyzing vapor phase samples and have relatively low instrumental resolution. During this project, our lab performed the following activities:

Developed a high-resolution ESI-IMS instrument for water sampling,

Tested several chemical warfare agent degradation products,

Mass identified the characteristic ions for the simulants with an IMS coupled to a quadrupole mass spectrometer,

Coupled an ESI-IMS to a time-of-flight mass spectrometer for rapid two-dimensional separations, and

Constructed a transportable ESI-IMS instrument.

The results of this work have led to an understanding of the mobility and mass spectra for chemical warfare degradation products and their required experimental conditions. In addition, our laboratory utilized the ESI-IMS-MS instrumentation to study several biological molecules (peptides, proteins and lipids). With the understanding gained from these preliminary studies, we may be able to use the instrumentation to analyze biological agents. The ability for one instrument to detect a wide range of agents would enable simpler detection systems. In addition, a field transportable system was constructed for environmental monitoring. This instrumentation may lead to a portable method that can directly analyze aqueous samples in near real-time.

SUMMARY OF THE MOST IMPORTANT RESULTS

Overview

Our work on this ARO project has shown that ESI/IMS can sensitively detect and analyze chemical warfare agents. A transportable ESI/IMS instrument was constructed based on results generated from work performed with our prototype instrument based on our previous studies. Also, an ESI/IMS/TOFMS was constructed as an additional analytical tool for the identification of chemical warfare agents. However, the abrupt termination of funding for this project prevented the construction of a portable instrument. In addition, due to cutbacks in funding, no field work was possible.

ESI/IMS/TOFMS instrumentation

An ESI-IMS-TOFMS instrument was constructed. The primary benefit of coupling an IMS with a time-of-flight mass spectrometer is that the IMS separates ions in milliseconds and the TOFMS generates mass spectra in microseconds, enabling two dimensions of sample information to be obtained very rapidly. Because the IMS operates at atmospheric pressure and the TOFMS operates at 10^{-6} Torr, a pressure interface comprising a series of lenses was required between the two separation techniques. The operational pressure in the interface is $\sim 10^{-2}$ Torr and manipulation of the lens voltages enables fragmentation of the ions. Including the fragmentation ions, there are three degrees of information; mobility spectra, whole molecule mass spectra, and fragmentation mass spectra. Furthermore, the flight tube in the MS was designed to be short in length and contains a reflector ion mirror to improve the MS resolution while maintaining a relatively small chamber. The smaller size MS combined with the use of a turbo pump has made this a benchtop instrument.

Verification of mobility data for CW agents with two independent instruments.

Previous preliminary studies were performed on our high resolution ESI-IMS with a quadrupole mass spectrometer (Asbury and Hill, 2000). Therefore, one of the initial evaluations with the new instrument was to compare mobility and mass spectral data for reproducibility and sensitivity. These studies were performed by two different graduate students with a separation of 1.5 years between experiments, ensuring that independent data was obtained for each instrument.

In IMS, the mobility values (standardized for temperature and pressure) as shown in the following equation are experimentally determined and utilized as an identification tool for the analytes of interest:

$$K_o = (L^2/Vt_d)(273.5/T)(P/760)$$

where L is the length of the drift tube, V is the voltage applied to the gate, t_d is the drift time of the ion, P is the pressure and T is the temperature. For comparison of the two ESI-IMS-MS instruments, the K_o values for each of the ions were compared for reproducibility. Because the values are standardized for temperature and pressure, K_o values using different instruments and experimental conditions can be compared and the deviation in values between instruments should be within 3%. Comparison of the two sets of K_o values showed excellent reproducibility and correlation between ion identities. In this study, the number of degradation products tested was modified to include the simulants recommended through the Chemical Warfare Convention.

Two-dimensional separation of CW simulants with IMS-TOFMS

Due to the time compatibility of the IMS (milliseconds) and the TOFMS (microseconds), two dimensions of information about an aqueous mixture can be ascertained, enabling confident identification of a possible CW agent or degradation product. In Figure 3 of Steiner et al., 2002, the two-dimensional separation of the degradation products discussed in Table 1 (Steiner et al., 2002) are shown. In addition, a series of calibrant ions (alkylamines) are also shown in the spectra. Since ion mobility actually separates based on the three-dimensional shape of the ions, the elongated alkylamines have longer drift times than the CW agents with the same mass. This is the reason that the alkylamines are clearly spatially resolved in the mobility/ mass to charge plot. Due to the lack of interference in the analysis, addition of an internal calibrant (one of the alkyl amines) can be utilized both as a m/z and mobility calibrant as well as for quantitation. Utilizing the alkyl amines as an internal standard for quantitation enables quantitation in the presence of different aqueous environments, providing slightly different analyte responses from the analytes of interest. From comparison of the mobility spectra for the CW degradation products, it can be seen that many ions either have the same m/z or drift time, but all are spatially resolved with the two dimensions of information. Although not all of the degradation products would be expected in a contaminated aqueous sample, it shows that the two dimensions of separation could be similarly utilized to separate other interferents (pesticides, insecticides, antibiotics) from the degradation products. Due to the implications involved in a positive ID for an agent or degradation product, the additional confirmation by MS is expected to be invaluable.

Design of the transportable ESI-IMS

Based on the experiments with the initial ESI-IMS prototype, a field transportable ESI-IMS was designed. The major components of the ESI-IMS were designed to fit within a case, including the drift tube, the control electronics, and the air pumps. The unit can be opened from both the back and front for maintenance and sample introduction. Air is supplied to the drift tube by pumps located at the bottom the case. Although funding to complete the project through September 2003 was promised, funds were not appropriated and we were informed in October of 2002 that the project had been terminated. At that time we had already incurred considerable expense for the final stage of the project and were forced to abruptly terminate the project and the portable instrument was not finished nor tested.

The studies with the prototype instrument showed that the ESI source was susceptible to overheating. In the prototype design, the ESI source was inserted approximately 2 in. into the mobility tube, but this was found to rapidly deteriorate the spectral quality. In the current design, the ESI source is placed at the very end of the tube and is connected to the hinged Teflon door for easy manipulation. This enables sufficient cooling of the ESI needle and access to the ESI source to routinely change the needles for optimal performance without significant disruption of the other instrumental components. Both insulating foam and Teflon are implemented as surrounding components of the IMS drift tube in order to ensure both thermal and electrical isolation since the mobility tube is operated around 200 deg C and 10,000 Volts.

LISTING OF PUBLICATIONS

Papers published in peer-reviewed journals

Asbury, G.R. and H.H. Hill, Jr., 1999, Negative ion electrospray ionization ion mobility spectrometry. *International Journal of Ion Mobility Spectrometry*, vol. 2, p. 1–8

Asbury, G.R., C. Wu, W.F. Siems, and H.H. Hill, Jr., 2000, Separation and identification of some chemical warfare degradation products using electrospray high resolution ion mobility spectrometry with mass selected detection. *Analytica Chimica Acta*, vol. 404, p. 273–283

Hill, H.H., Jr. and S.J. Martin, 2002, Conventional analytical methods for chemical warfare agents. *Pure and Applied Chemistry*, vol. 74, p. 2281–2291

Matz, L.M., B.H. Clowers, W.E. Steiner, W.F. Siems, and H.H. Hill, Jr., 2001, Liquid-sheath-flow electrospray ionization feasibility study of direct water analysis with the use of high-resolution ion-mobility spectrometry. *Field Analytical Chemistry and Technology*, vol. 5, p. 91–96.

Steiner, W.E., B.H. Clowers, K. Fuhrer, M. Gonin, L.M. Matz, W.F. Siems, A.J. Schultz, and H.H. Hill, Jr., 2001, Electrospray ionization with ambient pressure ion mobility separation and mass analysis

by orthogonal time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, vol. 15, p. 2221–2226.

Steiner, W.E., B.H. Clowers, L.M. Matz, W.F. Siems, and H.H. Hill, Jr., 2002, Rapid screening of aqueous chemical warfare agent degradation products: Ambient pressure ion mobility mass spectrometry. *Analytical Chemistry*, vol. 74, p. 4343–4352.

Papers published in non-peer-reviewed journals or in conference proceedings

None

Papers presented at meetings, but not published in conference proceedings

None

Manuscripts submitted, but not published

None

Technical reports submitted to ARO

The final report for the DURIP grant was submitted in 2001. The number of the grant is no longer available.

LIST OF PARTICIPATING SCIENTIFIC PERSONNEL

The following table is a list of all participating personnel showing any advanced degrees earned by them while employed on this project.

Name	Degree Earned
G.R. Asbury	PhD
B.F. Clowers	None
K. Fuhrer	None
M. Gonin	None
H.H. Hill, Jr	None
S.J. Martin	None
L.M. Matz	None
A.J. Schultz	None
W.F. Siems	None
W.E. Steiner	None
C. Wu	None

REPORT OF INVENTIONS

The report of inventions and subcontracts (Form 882) was submitted on 27 February 2003 by the Office of Grant and Research Development, Washington State University. No inventions were reported.

BIBLIOGRAPHY

Asbury, G.R., C. Wu, W.F. Siems, and H.H. Hill, Jr., 2000, Separation and identification of some chemical warfare degradation products using electrospray high resolution ion mobility spectrometry with mass selected detection. *Analytica Chimica Acta*, vol. 404, p. 273–283.

Steiner, W.E., B.H. Clowers, L.M. Matz, W.F. Siems, and H.H. Hill, Jr., 2002, Rapid screening of aqueous chemical warfare agent degradation products: Ambient pressure ion mobility mass spectrometry, *Analytical Chemistry*, vol. 74, p. 4343–4352